PROTEIN DEGRADATION OF BOVINE CALF SERUM IN SMALL AND LARGE DIAMETER METAL-ON-METAL HIP WEAR SIMULATIONS

*Webb J;**Bowsher, J G; ***Silwood C; ***Grootveld M; *+Shelton J C
*Dept. of Eng. and IRC in Biomedical Materials, Queen Mary, University of London, UK. +Email: j.shelton@qmul.co.uk

Statement of Purpose: Bovine calf serum (BCS) was recommended for laboratory wear testing of artificial joints by McKellop and Clarke in 1978 [1], and has become part of ASTM & ISO standards. However, nearly thirty years later there is still little consensus on BCS's characteristics and stability during long-term wear simulations.

Protein precipitation of BCS, as a result of articular frictional heating, can unintentionally protect prosthetic joints from wear [2]. Moreover, studies of Co-Cr on UHMWPE have reported rapid protein loss, with one study showing a 50% reduction in total protein after 1.0 million cycles [3]. To date, the stability of BCS in metal-on-metal (MOM) hip wear episodes has not been reported.

Therefore, the stability of BCS as a test lubricant was investigated using both small and large diameter MOM bearings. The test hypothesis was that smaller head sizes will show greater protein degradation. For the first time, nuclear magnetic resonance (NMR) spectroscopy was utilised to examine changes in BCS. ^{1}H NMR provides simultaneous multicomponent information regarding the metabolic status of biofluids.

Methods: 8 MOM bearings were studied: 4 of 28mm, 4 of 56mm, cast HC Co-Cr (Corin Medical, UK). Diametral clearances were 84 and 286μm for the 28 and 56 mm bearings, respectively. Test duration was 2.0 million cycles (Mc) of simulated walking in an orbital hip simulator (MTS, USA), using newborn calf serum (Sigma UK: diluted to 13 mg/ml protein). Water was added to compensate for lubricant evaporation. Serum samples were examined at 0–0.5 Mc for the running-in phase (RI), and 1.5–2.0 Mc for the steady-state phase (SS). Serum samples were collected every 24 hrs and frozen. A total of 82 samples were analysed. Fresh protein was used as a control. The total protein content was measured using a bicinchoninic acid protein kit (BCA: Perbio, UK). The samples were centrifuged to remove debris, then nuclear magnetic resonance (NMR) spectroscopy were conducted (Bruker-Avance 600).

Results: Both 28 and 56 mm MOM bearings exhibited high wear during the RI phase (Table 1), with the 56 mm bearings showing the greater wear. Both bearing sizes showed a reduced SS wear trend, with the 28mm bearings now showing the greater wear (Table 1).

Table 1	Running-In (0–1.0 Mc)		Steady-State (1.0–2.0 Mc)	
Diameter (mm)	**28**	**56**	**28**	**56**
Wear Rate (mm^3/Mc)	4.2	7.1	1.0	0.3

During the RI phase, serum from both ball sizes demonstrated a significant loss of protein content, dropping from 12.5 ±0.1 to 0.5 ±0.3 mg/ml by 56 hours (200,000 cycles: Fig. 1). After 80 hours of hip simulation (0.3 Mc), the protein content was almost zero. During the SS phase, all samples showed slower protein degradation, statistically different to RI phase ($p<0.05$). Loss of NMR-detectable biomolecules correlated with the higher wear running-in phase (Fig 2).

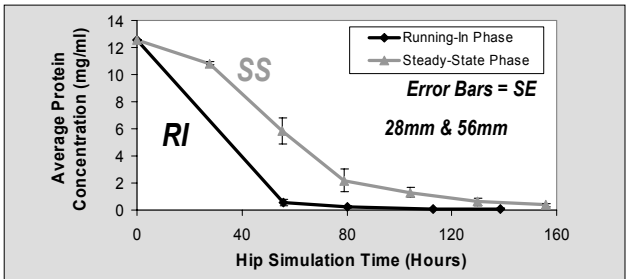

Figure 1. ***BCA*** *results: Typical drop in serum protein concentration vs time for both diameters under run-in and steady-state phases.*

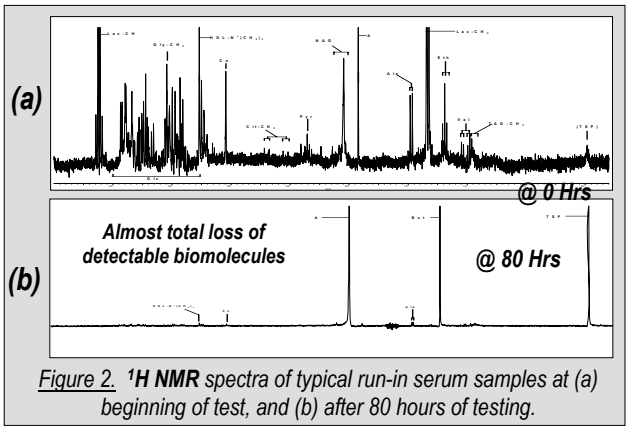

Figure 2. ***^{1}H NMR*** *spectra of typical run-in serum samples at (a) beginning of test, and (b) after 80 hours of testing.*

Discussion and Conclusions: Both BCA and NMR results confirmed that newborn calf serum was an unstable lubricant for both small and large diameter MOM hip joints. The rate of protein degradation in our MOM study was much greater compared to a study of Co-Cr-on-UHMWPE joints [3].

The influence of head size on protein degradation proved to be weak, even though there were significant differences in wear rate. These results suggested that rapid protein degradation only occurs as a result of higher wear, as seen during running-in (≥4.0 mm^3/Mc). Therefore, our test hypothesis was negated.

The results suggested that adding serum during a test, instead of water to sustain lubricant volume, may help to maintain protein content. There are also substantial grounds for the development of a more stable, physiologically relevant test lubricant.

References: [1] McKellop *et al*, JBMR 1978 Nov, 12(6). [2] Wang *et al*, JBMR, Part B, 68(B), 2004. [3] Yao *et al*, 28th Trans, Soc Biomat, 294, 2002. **Acknowledgements:** The authors thank Corin Medical for the supply of the components. **THE Dept. of Orthop., LLUMC, CA. & ***South Bank University, UK.